

Development of Novel Noninvasive Methods of Stress Assessment in Baleen Whales

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Award Number: N000141310639

http://www.neaq.org/conservation_and_research/projects/project_pages/right_whale_research.php

http://www.neaq.org/conservation_and_research/projects/conservation_medicine/index.php

http://www.neaq.org/conservation_and_research/projects/project_pages/marine_stress_research_program.php

LONG-TERM GOALS

Our long-term goal is to broaden the existing panel of endocrine stress assessment techniques for large whales. Few methods exist for assessment of physiological stress levels of free-swimming cetaceans (Amaral 2010, ONR 2010, Hunt et al. 2013a). We have previously demonstrated that respiratory vapor (blow) sampling is practical and feasible for large whales, and that blow samples contain detectable steroid and thyroid hormones (Hunt et al. 2013b). We have also developed a suite of fecal hormone assays for reproductive and stress-related hormones in North Atlantic right whales (Hunt et al. 2006, Rolland et al. 2005, 2012). However, blow sampling needs further testing before it can enter widespread use, and some additional stress-related hormones have not yet been tested in either feces or blow, particularly aldosterone. Our aim in this project is to further develop both techniques - respiratory hormone analysis and fecal hormone analysis - for use in stress assessment of large whales.

OBJECTIVES

We have two specific objectives in this project: (1) further development of respiratory sampling methodology, via modifications to our sampling apparatus and testing of "internal controls" to control for water content; and (2) development of a noninvasive aldosterone assay (for both feces and blow) that can be used as an alternative measure of adrenal activation to complement existing glucocorticoid assays.

APPROACH

Though this project was scheduled to begin in March 1, 2013, we did not receive funds until mid-August, 2013. With only a few weeks left in the field season and in the FY2013 fiscal year, we scaled back our FY2013 approach to the following:

Report Documentation Page			Form Approved OMB No. 0704-0188		
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1. REPORT DATE 30 SEP 2013	2. REPORT TYPE		3. DATES COVERED 00-00-2013 to 00-00-2013		
4. TITLE AND SUBTITLE Development of Novel Noninvasive Methods of Stress Assessment in Baleen Whales			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) New England Aquarium,Central Wharf,Boston,MA,02110			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 8	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

Task 1, Fieldwork for Blow Sample Collection -

- (1) Hiring and training of a full-time postdoctoral researcher with expertise in development of noninvasive hormone assays and marine mammal field research. This post-doc will focus full-time on this project, enabling us to devote sufficient time and expertise to necessary R&D.
- (2) Modifications to blow sampling apparatus. We have a blow sampling apparatus (pole + pole mount) from our pilot study (FY2011-12). Based on prior experience with this pole, we wished to do minor modifications to this apparatus to achieve better maneuverability and better balance.
- (3) Development of novel respiratory sampler designs. In our pilot study (FY2011-12) we successfully tested a "bottle-and-veil" style sampler consisting of a plastic bottle wrapped with nylon veil. This sampler performed well, but is bulky and requires a great deal of processing time per sample. In the current grant we wish to test alternative sampler designs that might be less bulky and/or allow more rapid processing time.
- (4) Reorganization and minor repairs to field laboratory, to create a suitable space with which we can perform hormone assays on-site during the FY2014 and FY2015 field seasons.

Task 2c, Laboratory Validations - Aldosterone assay research. A major aim of this project is to develop and validate an aldosterone assay for baleen whales. The first step involves researching all commercially available aldosterone assays that are available in the US, as well as contacting other wildlife endocrinologists who may have unpublished information on aldosterone assays that work well for noninvasive matrices (e.g., feces, blow).

Key individuals in this project are: PI Kathleen Hunt, Ph.D. (fieldwork, analyses, reports and general oversight); Co-PI Rosalind Rolland, D.V.M. (fieldwork, experimental design and data interpretation); Co-PI Scott Kraus, Ph.D. (pole design/construction, boat piloting and other field logistics); and post-doctoral researcher Elizabeth Burgess, Ph.D. (fieldwork, laboratory analyses, R&D of novel lab analyses, data interpretation and analysis).

WORK COMPLETED

Task 1: Fieldwork for Blow Sample Collection

(1) Postdoctoral researcher hired and trained. In August 2013 (immediately upon receipt of FY2013 funds) we hired Elizabeth A. Burgess, Ph.D. Dr. Burgess will focus full-time on this grant throughout the duration of funding, will participate in all fieldwork and all labwork, and will take the lead on R&D of new laboratory analyses as well as data analysis. Dr. Burgess has years of experience with marine mammal fieldwork (including cetaceans) and has recently completed a Ph.D. on fecal hormone assay development for dugongs. In August 2013 Dr. Burgess received her US work visa, moved from Australia to Boston, MA. In September 2013 Dr. Burgess traveled with our team to Lubec, ME, to receive orientation and training for Bay of Fundy boat-based fieldwork, including safety training onboard R/V *Callisto*, and participation in Bay of Fundy marine mammal surveys.

(2) Modifications to blow sampling apparatus and sampler design. Based on experience in our FY2011 pilot project, we have constructed and attached a rectangular pole handle that provides better pole control, and also a longer pole mount that makes the pole much better balanced.

(3) Development of novel respiratory sampler designs. We have constructed two novel styles of respiratory samplers to compare to our existing bottle-and-veil design: (1) "Hoop-and-veil" samplers, consisting of 2m² of nylon veil stretched across a custom-built stainless steel hoop (similar to an embroidery hoop in design; Figure 1). (2) "Plate" samplers. In FY2011 we discovered that a spray of blow droplets can adhere to a plexiglass plate via surface tension, and that droplets can potentially be recovered with a squeegee or pipette. In FY2013 we refined this method by means of using laboratory-grade sterile polystyrene plates that can be attached to a velcro attachment at the end of the pole. (Figure 2).

(4) Completed field lab inventory and repairs. In preparation for FY2014 fieldwork we have inventoried and reorganized our field lab, completed multiple repairs to cabinet doors, drawers, window frames, and floor. The lab now has suitable space in which we can perform enzyme immunoassays (EIAs) and other analyses on fresh blow droplets during the FY2014 and FY2015 field seasons.

Task 2a: Lab Validations - Sampler Testing for Blow

This task will occur in FY2014. We plan to begin with laboratory trials of percentage recovery of hormones from blow samplers, by spraying known doses of hormones (in varying ratios) onto all of our types of samplers (bottle with veil, hoop with veil, and plate) and then processing each sampler to extract and measure hormone quantities retained by the sampler. This experiment will provide us with information to compare the efficiency of hormone recovery of each sampling apparatus.

Task 2a: Lab Validations - Development of an Internal Control for Blow

This task will occur in FY2014 and FY2015. In early FY2014 our major objective is to test several potential internal controls, initially focusing on measures of albumin, total protein and total lipids. This early testing will use remaining archived blow samples from NARW (e.g. samples that were collected in 2011 in our pilot study), potentially supplemented with humpback blow samples if needed. We have at hand over 50 fresh humpback blow samples collected in 2013 which were archived under another project; these blow samples were used only for preliminary parallelism testing of 3 hormone assays, and the remaining aliquots and samples can be made available for this grant, e.g. getting internal-control testing underway for blow samples from a species of baleen whale. Preliminary validation studies using humpback blow samples would also be verified using right whale blow samples.

Task 2c: Lab Validations - Development of a Fecal Aldosterone Assay

We have begun research on aldosterone assays by surveying all available commercial aldosterone assays (both RIA and EIA) in the USA. We have also consulted with other ONR marine mammal researchers and with endocrinologists of the International Society for Wildlife Endocrinology. Based on this information we have selected three aldosterone kits for initial testing with NARW fecal extracts in early FY2014. The three kits will include a Siemens Coat-a-Count kit that performs well with cetacean serum (D. Crocker, pers. comm.) along with two other kits that require low sample volume and have good precision and reproducibility. Kit testing will begin in FY2014 with standard parallelism and accuracy tests on sample pools of fecal extracts of known-sex right whales. We will then assay all archived NARW fecal samples for aldosterone.

Task 2d: Assaying New Blow and Fecal Samples for Stress and Reproductive Hormones

This task will occur in FY2014 and FY2015, with fieldwork planned for both years. Note that due to the delay in receiving funds this year, most of our FY2013 fieldwork had to be postponed to FY2014 and FY2015.

Task 3: Data Analysis, Manuscript Preparation, and Reporting

This task will occur in FY2014, FY2015 and FY2016. We expect to produce at least two publications, one on fecal aldosterone and one on respiratory vapor analyses.

RESULTS

As this grant has only been underway for six weeks, we have not yet begun the bulk of our fieldwork. Due to the delay in receiving funds, most fieldwork was rescheduled for FY2014 and FY2015. We did achieve two days at sea at the very end of FY2013 (late September), but due to extremely low whale abundance in FY2013 and limited field days, we used this time for testing our new pole and sampler designs in the field, preparing the lab at our field station, and training new team members on boat safety and pole operation.

Our primary results to date are therefore the modifications to our respiratory-sampling pole apparatus, development of two new sampler types ("hoop-and-veil" and "plate" samplers; Figures 1, 2), training of our boat crew, and selection of aldosterone assay kits for initial testing.

Our work in FY2014 will focus on (1) aldosterone assay development (parallelism and accuracy tests, final selection of assay kit, and assay of all archived NARW fecal samples), and (2) internal-control development for respiratory samples (using archived NARW blow samples, potentially supplemented with archived humpback blow samples).



Figure 1. "Hoop" style sampler in close-up (top) and attached to pole (bottom). A nylon veil can be stretched across the stainless-steel hoop, and a quick-release pin (visible in top photo) allows rapid interchange with other devices.



Figure 2. "Plate" style samplers. Top, sterile laboratory-grade polystyrene plate with velcro patch attached to back; Bottom, view of plate attached to pole with velcro.

IMPACT/APPLICATIONS

This project will refine and test sampling techniques for collection of respiratory vapor from large baleen whales, as well as develop a novel assay for a stress-related adrenal hormone (aldosterone) that has not been adequately studied in baleen whales. Respiratory sampling is a novel method of physiological assessment for large whales that may offer the unparalleled ability to rapidly collect

repeated, noninvasive samples from targeted individuals with minimal disturbance (Hunt et al. 2013a, 2013b). Further development of this sampling technique could enable the Navy to apply novel tools for monitoring the effects of disturbance on cetaceans on a population-wide basis. Fecal sampling has already been demonstrated to be an effective tool for assessing physiological stress in cetaceans (Rolland et al. 2012), but there is a need for additional measures of adrenal activation that can complement existing glucocorticoid measures. Aldosterone, the other steroid that is released by the adrenal gland, may offer such a complementary measure and will further characterize the physiological responses of marine mammals (Ortiz 2001, St. Aubin 2001). Together, the two analytic techniques - respiratory analyses and fecal analyses - could help assess physiological stress at different timescales after disturbance, with respiratory analyses reflecting short-term disturbances and fecal analyses longer-term disturbances (Hunt et al. 2013a). These new analyses may provide novel tools needed to evaluate the physiologic consequences of disturbance on baleen whales.

RELATED PROJECTS

ONR award # N000141110540, "Assessing stress responses in beaked and sperm whales in the Bahamas" (PI Rolland), also focuses on development of novel methods for physiological stress assessment in cetaceans. That project focuses on fecal hormone assays that have already been well-validated for North Atlantic right whale (e.g. glucocorticoids, thyroid hormones, progestins, androgens, estrogens), the aim being to validate those hormone assays for application in other species that have varying exposure to acoustic-related disturbance.

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